Reduction of analysis times in HPLC at elevated column temperatures

H.A. Claessens[1,2] and M.A. van Straten[1]

1. Eindhoven University of Technology, Department of Chemical Engineering and Chemistry, Research group of Polymer Chemistry, Eindhoven, the Netherlands
2. Avans University of Professional Education, Department of Life Sciences, Breda, the Netherlands

Contents of this lecture

- Four major practical questions
- Sample throughput, Analysis time reduction
- Conclusions / remarks

Four major practical questions

- How to select the optimal column for a specific separation from the several hundreds available, and in many cases, nominally identical, RP-columns?
- How to perform an objective and fast method development procedure resulting in an optimal and rugged analysis protocol, Sample throughput?
- Upon validation of an RPLC analysis protocol, what will be the repeatability and reproducibility of columns and batches of a specific stationary phase guaranteeing an undisturbed continuous analysis process.
- Once column and separation method have been worked out, what will be the longevity of that column under the applied conditions; In other words, how many sample injections can be performed in a column life cycle time.

Analysis time

Why faster analysis ?

- Faster QC, process control and other analysis
- Handle more complex samples without increasing analysis time
- Rapid method development
- Reduce solvent use/disposal
- Reduce costs
- Improves total productivity

Analysis time

Analysis speed can be substantially improved

- Ultra high pressure liquid chromatography (UPLC)
- Capillary electro chromatography (CEC)
- Monolithic columns
- Increasing column temperature

Analysis time

Requirements:

- Columns of high permeability
- Short packed columns: 50 mm or shorter; Small particle sizes; 3.5 µm or smaller: 1.8 µm
- Monolithic columns
- Optimized HPLC equipment; eliminating extra-column band broadening effects; tubing, detector cell, pumps delivering increased pressure and/or eluent flows, well designed thermostatting systems
- Electronics; detector response time – data sampling rate

Analysis time

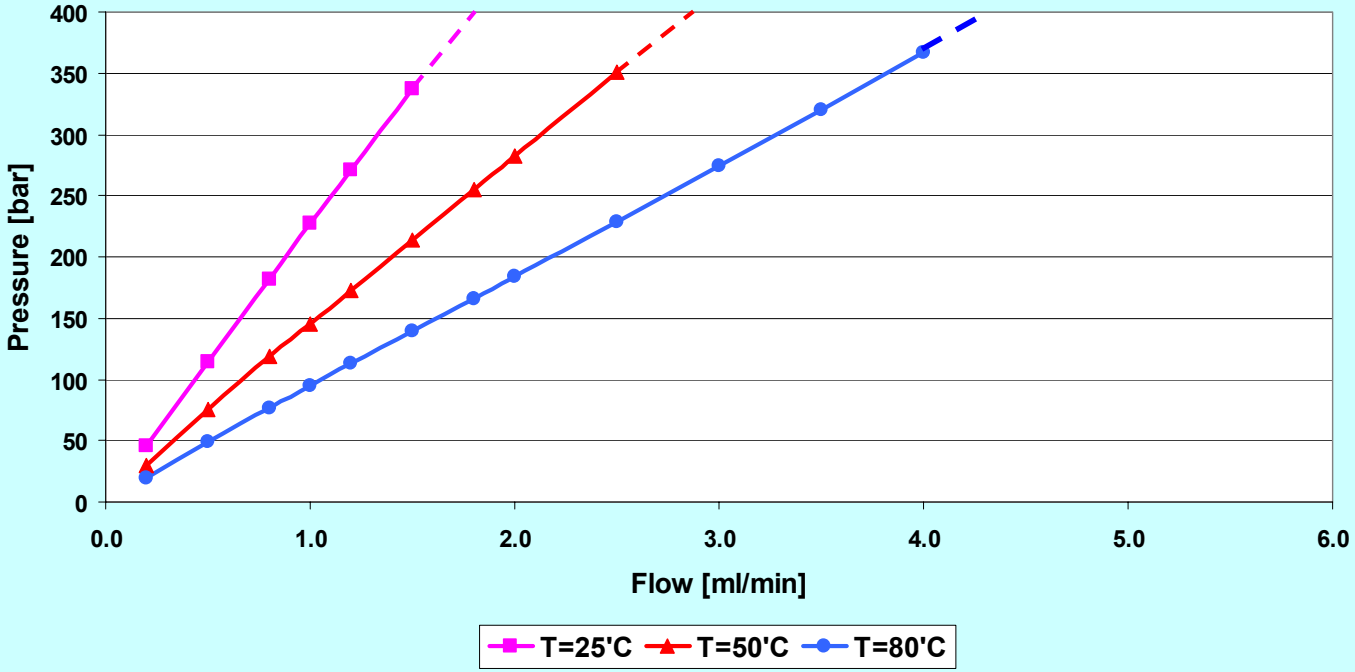
Analysis at increased temperature require:

- thermally stable stationary phases
- thermally stable analytes
- optimized HPLC equipment
- knowledge of influence T on retention and selectivity

Analysis time

dp = 1.8 µm

Eluent: Methanol/Water = 60/40 (v/v)



Analysis time

Column: SB-C18, 4.6 x 50 mm; 1.8 µm

$$\frac{dF}{dP} = \left(ml/100bar.\min\right)$$

Eluent: Methanol / Water = 60/40 (v/v)

25°C	50°C	80°C
0.45	0.72 (1.6)	1.10 (2.4)

Eluent: Acetonitrile / Water = 50/50 (v/v)

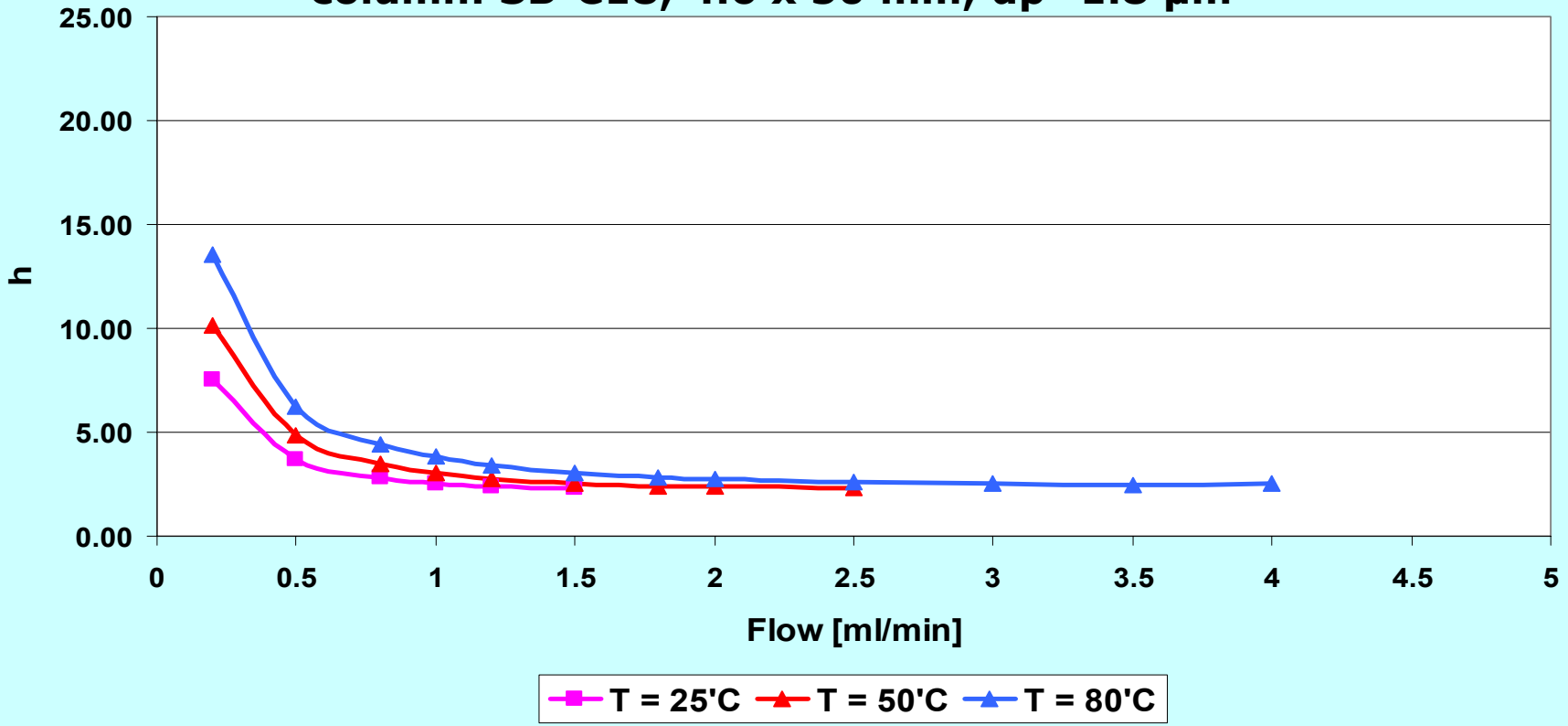
25°C	50°C	80°C
0.77	1.11 (1.4)	1.52 (1.9)

Analysis time

h-flow curves [calculated from N Gauss]

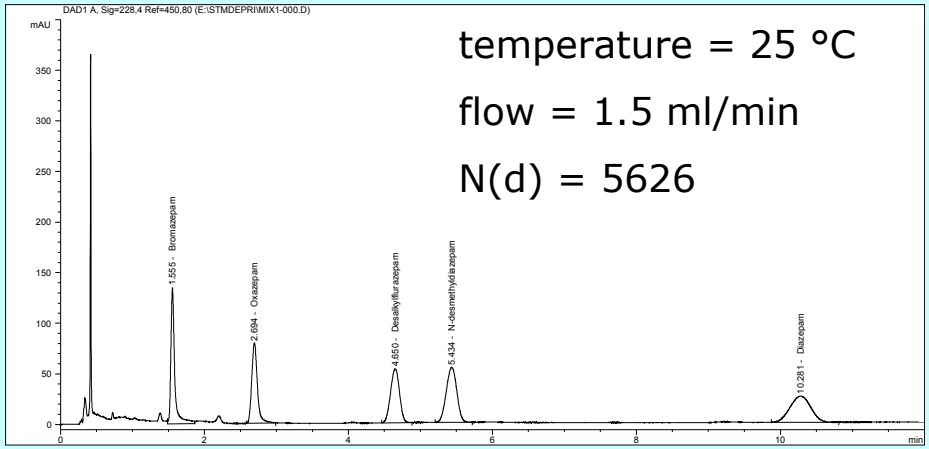
Methanol / Water = 60/40 (v/v)

column: SB-C18; 4.6 x 50 mm; dp=1.8 µm



Application examples

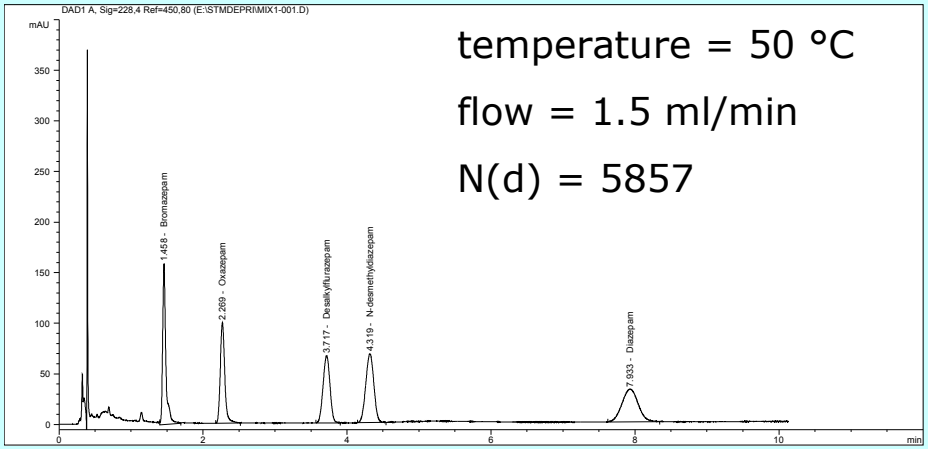
Separation of Sedativa



temperature = 25 °C

flow = 1.5 ml/min

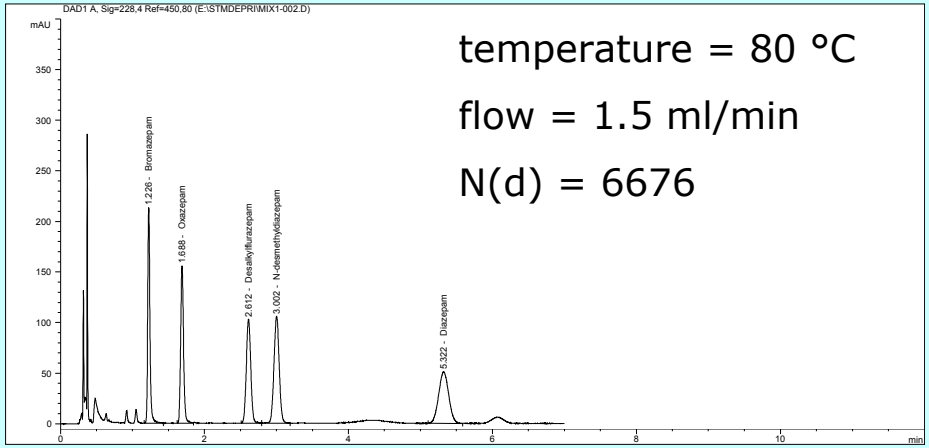
N(d) = 5626



temperature = 50 °C

flow = 1.5 ml/min

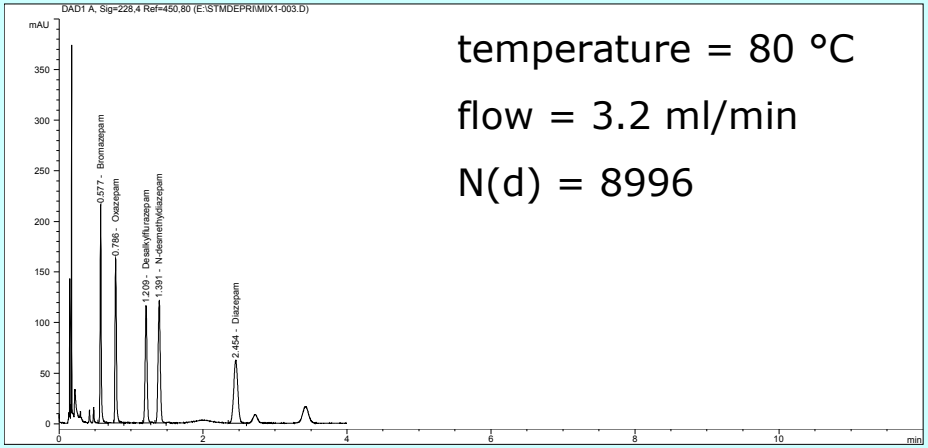
N(d) = 5857



temperature = 80 °C

flow = 1.5 ml/min

N(d) = 6676



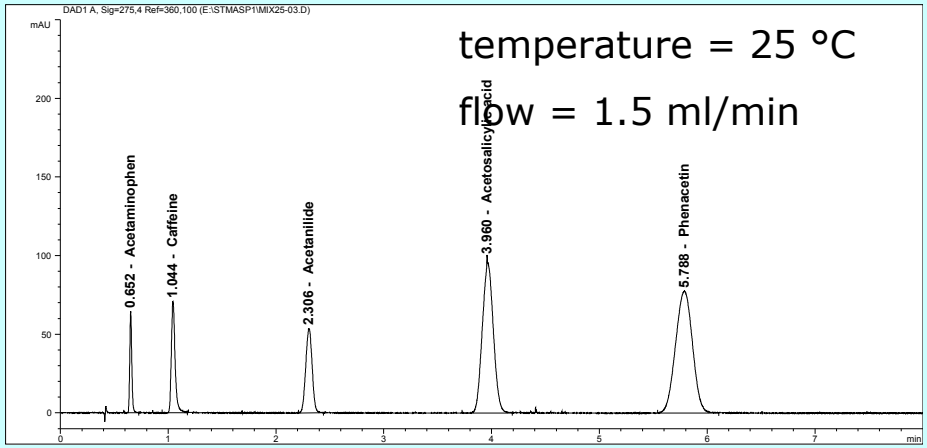
temperature = 80 °C

flow = 3.2 ml/min

N(d) = 8996

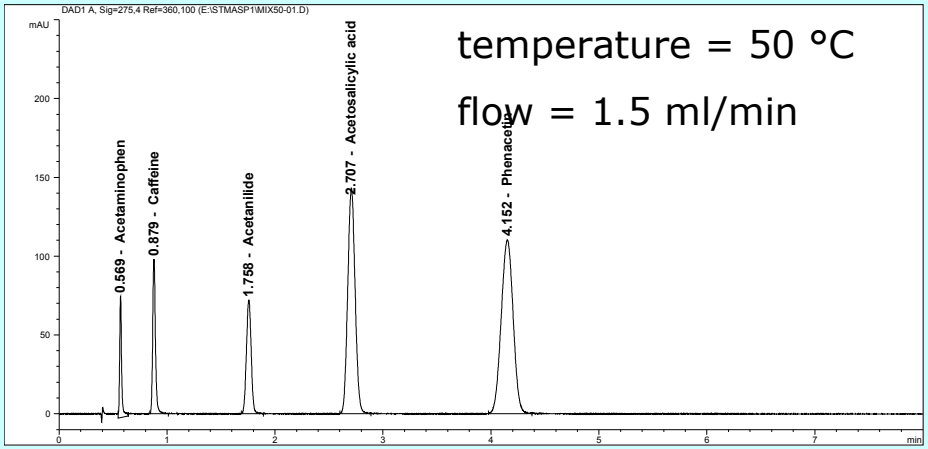
Application examples

Separation of Analgetics



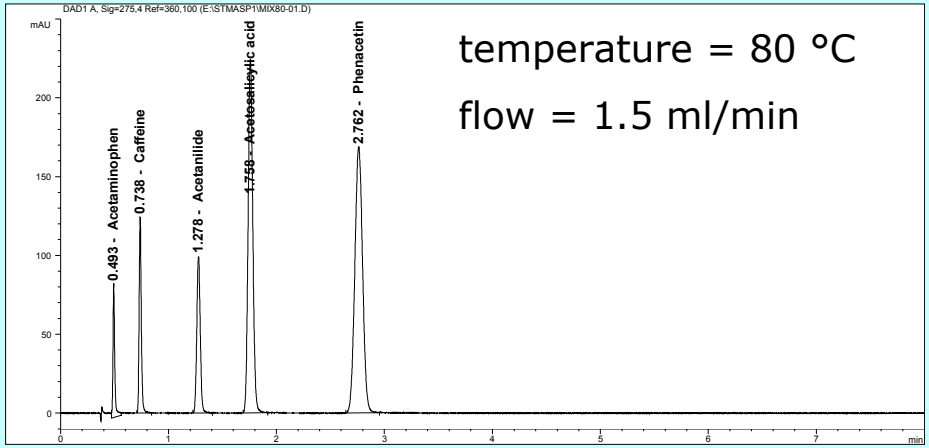
temperature = 25 °C

flow = 1.5 ml/min



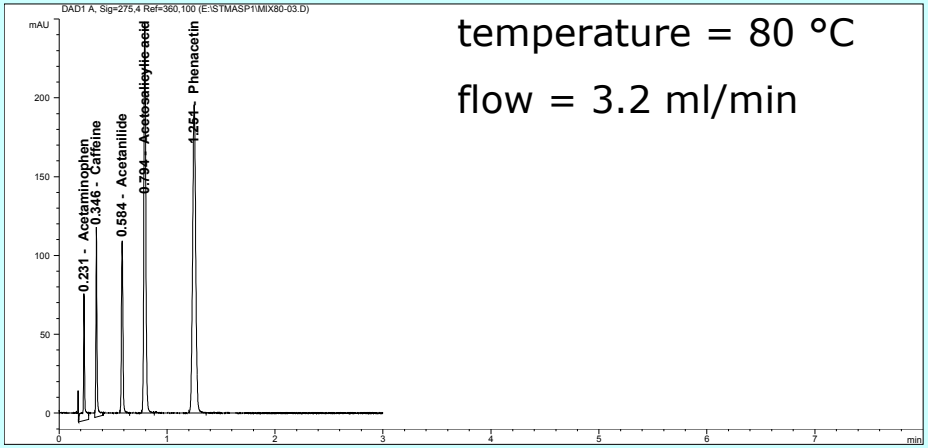
temperature = 50 °C

flow = 1.5 ml/min



temperature = 80 °C

flow = 1.5 ml/min

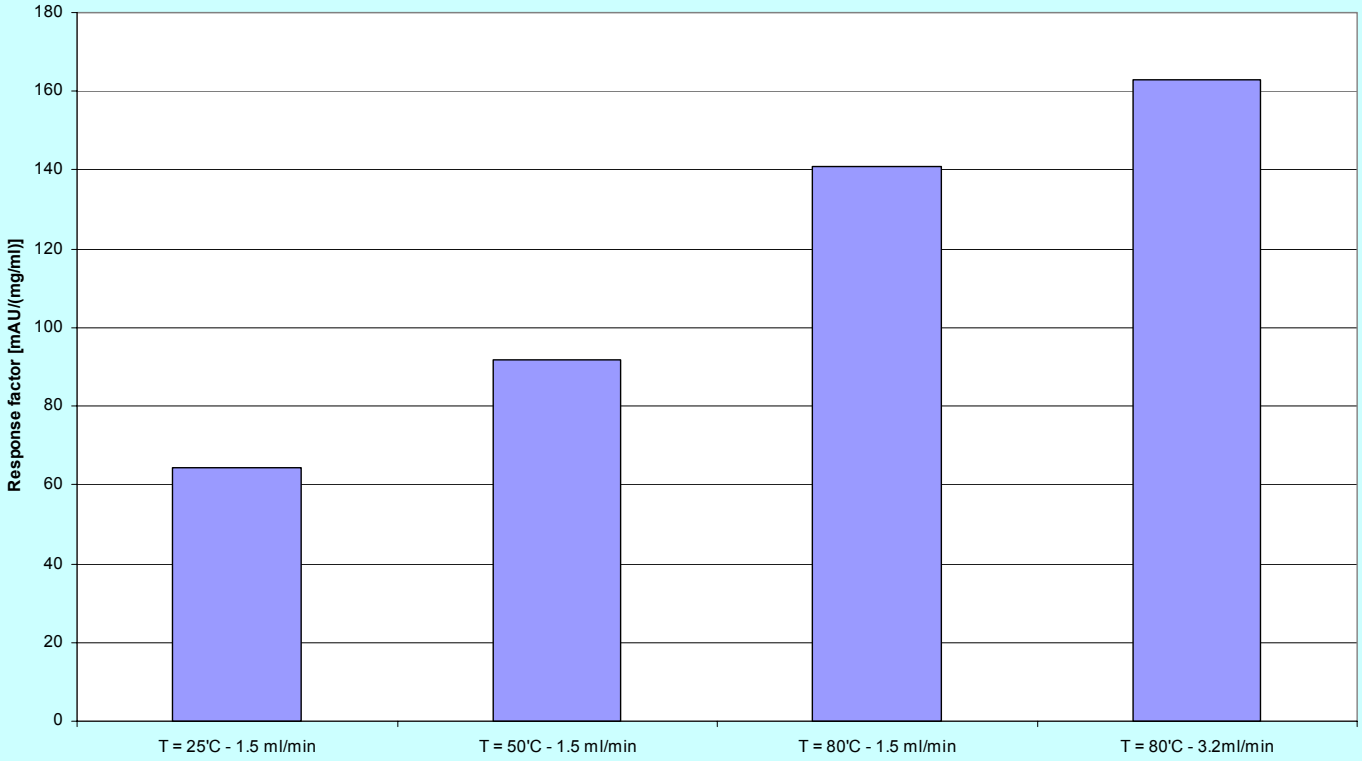


temperature = 80 °C

flow = 3.2 ml/min

Application examples

Response factor for Phenacetin



Conclusions / remarks

- Upon temperature increase combined effects of cubical liquid expansion, decreasing eluent viscosity and increased analytes diffusion are involved
- Up to 80 °C analysis time can be reduced by a factor of about 4; conventional equipment
- Requirements: columns and analytes (dynamic) must be thermally stable

Conclusions / remarks

- Chemically and thermally stable RPLC phases based on silica (90 °C); other inorganic oxides (190 °C) and polymers (150 °C) are available

- Apart from UPLC, CEC and monoliths also temperature is a strong tool to reduce analysis times

Acknowledgement

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